Developmental instability as an index of adaptation to drought stress in a Mediterranean oak

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25 Abstract

26 An increase in temperature and water deficits caused by the ongoing climate change
27 might lead to a decline growth rates and threaten the persistence of tree species in
28 drought-prone areas within the Mediterranean Basin. Developmental instability (the
29 error in development caused by stress) may provide an index of the adaptability of
30 woody plants to withstand climatic stressors such as water shortage. This study
31 evaluated the effects of drought stress on growth variables in three stands of a
32 Mediterranean oak (*Quercus faginea*) exposed to differing climatic conditions (xeric,
33 mesic and cooler) along an altitudinal gradient in northeastern Spain, in two climatically
34 contrasting years (wet and dry years). Two indices of developmental instability,
35 fluctuating and translational asymmetries, which reflect environmental stress, were
36 measured in leaves and current-year shoots respectively. We also measured branch
37 biomass and fractal complexity of branches as indicators of the species' performance.
38 After a period of drought the individuals' at the most xeric site presented lower
39 developmental instability and less branch biomass than did the individuals from the
40 mesic and cooler sites. We interpret that difference as an adaptive response to drought
41 which reflects a trade-off between maintenance of homeostasis and growth when water
42 is scarce. The study demonstrated that developmental instability constitutes a useful
43 index to assess the degree of adaptation to stressful environmental conditions. The
44 assessment of developmental instability in sites and years with contrasting climatic
45 conditions provides a means of quantifying the capacity of plants to develop plastic
46 adaptive responses to climatic stress.

47

48 *Key words*: developmental instability; drought stress; fractal dimension; Mediterranean
49 oak; phenotypic plasticity; adaptation.

1. Introduction

Water availability, high temperatures and radiation are among the most important environmental constraints for plant growth and persistence in Mediterranean ecosystems (Chaves et al., 2003; Zunzunegui et al, 2000). Climate models have predicted increases in temperature and frequency of severe drought events in the Mediterranean Basin (Bates et al., 2008; Giorgi and Lionello, 2008; Luterbacher et al, 2004). Furthermore, several studies reported reductions in precipitation in some Mediterranean areas as the Iberian Peninsula (e.g., Rodriguez–Puebla and Nieto, 2010). Such increases in aridity have negative consequences for plant performance in those drought–prone areas (Walther et al, 2002). It is important to understand the responses of plants to drought in those areas in order to predict the possible changes in the natural vegetation in response to global warming. Those responses might include adaptations that involve phenotypic plasticity, which is the capacity of organisms to express alternative phenotypes in response to environmental variation (Schlichting, 1986). Plasticity is one of the most important short–term mechanisms used by plants to cope with rapid environmental change (Ramirez–Valiente et al., 2010; Voesenek and Blom, 1996). A high adaptive phenotypic plasticity might permit populations to persist and adjust to climatic variability (Lindner et al, 2010).

Measurements of developmental instability (DI) can be used to quantify the phenotypic plasticity of plants. Traditionally DI has been used as index of stress (Moller and Swadel, 1997; Polak, 2003), due to being correlated to several biotic and abiotic stressors, including environmental factors such as interspecific competition (Komac and Alados, 2012), drought (Escós et al., 2000; Fair and Breshears, 2005), high temperature (Llorens et al., 2002), elevation (Hagen et al., 2008), radiation (Oleksyk, et al., 2004), herbivory (Moller, 1995; Escós et al., 1997; Puerta–Piñero et al., 2008); and

anthropogenic activities, such as habitat perturbation resulting of military practices, urbanization and pollution (Freeman et al., 2004; Cuevas–Reyes et al., 2013; Velickovic and Savic, 2012, respectively); as well as genetic factors such as mutation, inbreeding and hybridization (Hochwender and Fritz, 1999). DI is based on the hypothesis that as stress increases the ability of the plant developmental program to resist perturbations decreases (Freeman et al., 2004). Under stressful conditions the mechanisms that are intended to insure the correct development are interrupted leading to developmental errors (Freeman et al. 2003). Organisms are never perfectly symmetrical, however, and there is always certain degree of asymmetry, which is caused by developmental noise (DN), the small cumulative random errors in development caused by the stochasticity in cellular processes; DN increases as external stress does (Lens et al., 2002). Organisms have developed mechanisms to buffer against those developmental errors, referred to as developmental stability (DS), an individual ability to produce a predetermined invariant phenotype under particular environmental conditions (Moller and Shykoff, 1999; Polak, 2003). Thus, DS is the internal force which buffers against the errors in development manifested in DN, and DI is the combined result of the balance between the counteracting effects of DN and DS (Lens et al, 2002). Environmental stress can affect development by increasing DN, or by decreasing DS (Lens et al, 2002). If an organism is well adapted to a harsh environment it might have low DI because DS counteracts the increase in DN caused by environmental stress. High DS under stressful environmental conditions reflects that an organism is well adapted to such conditions. The subtle interplay between these three concepts is essential to the sound interpretation of the studies of developmental instability (Supporting information S1). Some studies demonstrated unclear relationship between DI and stress (Auslander et al., 2003; Duda et al., 2003; Fair and Breshears, 2005) or a negative correlation (Hódar, 2002). Those

differences might have occurred because some populations have adapted to certain degree of stress (Alados et al., 1999; Kaligaric, 2008; Velickovic and Saivc, 2012). Several authors also suggest that DI might serve as index of adaptation (Graham, 2010; Jones, 1987). In this study, DI was used as an index of adaptation rather than as an index of stress. DI in plants can be quantified in several ways, we use fluctuating asymmetry (bilateral symmetry) and translational asymmetry (based on allometric relationships). In addition we assessed the fractal complexity of the branches because fractal dimension can be an efficient indicator if stress in plants (Alados et al., 1998a, 1999; Escós et al., 2000).

This study evaluated the phenotypic plasticity of a Mediterranean oak *Quercus faginea* across a climatic gradient in two years that had contrasting climatic conditions. The spatio–temporal variation in climatic conditions represented by the climatic gradient, which included a xeric, a mesic and a cold site and the two years of study, provided a system in which DN might be enhanced by an increase in environmental stress, which might lead to an increase in DI. If, however, the trees are well adapted to their environment, the buffering capacity of plants, here assessed as DS, might compensate for any increase in of developmental error.

Our general objective was to assess the adaptive capacity of *Q. faginea* under climatically contrasting conditions which would help in predicting the response of this specie to the warmer and drier conditions forecasted for this region. Specifically we aimed: (1) to estimate the variation in DI of *Q. faginea* in xeric, mesic and cold environments in two climatically contrasting years (wet *vs.* dry conditions); and (2) to assess the relationship between shoot length and DI. Based on theoretical considerations we predicted that individuals at the most xeric site were adapted to semi–arid conditions and, therefore would have less DI after a dry year because they were better adapted to

drought than were the individuals subjected to humid conditions in the most mesic site. We expected to find a trade-off between shoot length and maintenance of DI, as a measure of the ability of the tree to maintain a stable development at expense of biomass production, particularly at the most xeric site.

2. Material and methods

2.1. Study area and species

The study area is located at the province of Huesca, in north-eastern Spain. The sampling sites included three locations along an altitudinal gradient: a xeric site in the Sierra de Alcubierre (Alcubierre site) of the Middle Ebro Basin, and two additional sites in the central Pre-Pyrenees (sites Arguis –**mesic site**– and Pico del Águila –**cold site**) which were visited in September and October of 2008 and 2009. The studied altitudinal gradient reflected a marked climatic gradient that was characterized by a decrease in temperature and an increase in precipitation upwards (Table 1 and Supporting information S3; for more information on climatic gradient see Alla and Camarero 2012).

Quercus faginea Lam. is a winter-deciduous Mediterranean oak widely distributed in the Iberian Peninsula in relatively humid areas with basic soils (Castro et al., 2005). The climatic conditions that influence shoot and leaf development are those that occur in the previous year (Chaubert-Pereira et al., 2009; Montserrat-Martí et al. 2009), in our study from August in 2007 until August in 2008 for the sampling year 2008 and from August 2008 until August 2009 for the sampling year 2009, because bud meristems are formed one year before shoot elongation and leaf expansion (Alla et al., 2011). In 2007, annual precipitation in the study area was lower than the mean for the reference period (1960–2006) ", **which**, for the purposes of our sampling, meant that

149 **2008 was a 'dry' year**. In 2008, precipitation was slightly higher than the average
150 **therefore the sampling year 2009 was a 'wet 'year** (Table 1).
151 *2.2. Field sampling and laboratory methods.*
152 At each of the three sites, ten *Q. faginea* mature individuals that were at least 5
153 m apart were chosen randomly on each of two transects. The diameter at a height of 1.3
154 m of the thickest stem of all sampled trees was measured. Shoot and leaf samples were
155 collected from the southward and the upper third of the crown. To quantify translational
156 asymmetry three current–year shoots were collected from each tree, and to measure
157 fluctuating asymmetry three current–year leaves were randomly selected from each of
158 these shoots. In addition, to quantify fractal complexity a five–year–old branch was
159 collected from each tree.
160 To calculate the translational asymmetry an electronic calliper (resolution 0.01
161 mm) was used to measure the internode length from the base to the top of each shoot
162 (Fig. 1). To estimate fluctuating asymmetry a 4800–dpi resolution scanner (Epson
163 Perfection 4990 Photo, Seiko Epson Corporation, Japan) was used to take a digital
164 photograph of each leaf, and the symmetry measurements were made using the image
165 analysis software Image Pro–Plus ver. 5.0 (Media Cybernetics, Bethesda, MD). In each
166 leaf we measured the distance from the central vein, here considered as the symmetry
167 axis, to both lateral edges of the leaf at three equidistant points along the axis of
168 symmetry (Fig. 1). To calculate the measurement error measurements were taken twice
169 (Swaddle et al., 1994).
170 Fractal complexity, quantified by information fractal dimension (IFD), was
171 calculated from digitized pictures of each 5–year old branch. The images were captured
172 at a uniform distance and just after the branches were collected. The dry weight of each
173 branch was recorded after it had been oven dried to a constant weight at 60º C.

174

175

176 *2.3. Statistical analyses*

177 2.3.1. Fluctuating asymmetry (FA)

178 The validity of fluctuating asymmetry as an estimate of environmental
179 perturbation requires the absence of directional symmetry and antisymmetry (Palmer
180 and Strobeck, 1986). Fluctuating asymmetry differs from the later two because the
181 values of left minus right sides (*L–R*) follow a normal distribution with a mean of zero.
182 The *L–R* distribution that differs from ideal fluctuating asymmetry is not a suitable
183 descriptor of developmental instability because some of the asymmetry might have a
184 genetic basis (Palmer and Strobeck, 1992). The distribution of the signed *L-R*
185 differences was evaluated using a Kolmogorov–Smirnov (K–S) Normality Test. To
186 assess the statistical significance of the fluctuating asymmetry, we used a mixed–model
187 (Two–way ANOVA) that included 'side' as a fixed–effect factor, which reflected
188 directional asymmetry, 'individual' as a random factor, which reflected the variation
189 among individuals, and a 'side–by–individuals' interaction term, which reflected
190 fluctuating asymmetry. (Palmer and Strobeck, 1986). The measurement error was
191 calculated as follows:

192 $$MSE = \frac{MS_{error}}{MS_{individual}} \times 100 \qquad (1)$$

193 where *MS error* is the mean square of the error term in the general model of
194 ANOVA and *MS individual* is the mean square of individual of type III. In the event
195 that there might be a relationship between the asymmetry measurements and leaf size
196 and therefore a need to normalize |*L–R*|, we performed a correlation between absolute
197 fluctuating asymmetry, |*L–R*|, and the leaf size, *(L+R)*. This correlation was highly
198 significant (r=0.52, p=0.0001), therefore the raw data were transformed using

199 logarithm. Besides, in order to deal with |*L*–*R*| half–normal distribution we applied the
200 Box–Cox transformation (following the recommendations of Swaddle et al. 1994;
201 Graham et al. 1998 and Freeman et al., 2004). We used FA as a global index of leaf
202 responses to stress and it was calculated as the sum of the three measures taken from
203 each leaf.

204 $$FA = \sum_{i=1}^{3} \left(\left| \ln L_i - \ln R_i \right| + 0.00005 \right)^{0.33} \qquad (2)$$

205 2.3.2. Translational asymmetry (TA)

206 Translational asymmetry was measured as the error in the following curve–
207 fitting equation:

208 $$L(N) = kN^{a}e^{-bN} \qquad (3)$$

209 where *L* is the internode length, *N* the internode order (measured from the bottom to the
210 top, see Fig. 1), *e* the natural base and *k*, *a* and *b* are the fitted parameters (Alados et al.,
211 1998b, 2006; Freeman et al., 2004; Tan–Krisanto et al., 2003).

212 Curve–fitting accuracy and parameter values were obtained after log–log
213 linearization and posterior linear regression adjustments for each plant. The coefficient
214 of determination, R^2, was used as translational asymmetry index (TA), as a measure of
215 the degree of developmental instability. A high coefficient of determination, which
216 corresponds to a good curve fit, indicates low DI. The parameters *a*, *b*, and ln *k* were
217 used to quantify the primary growth processes that occur during shoot elongation. The
218 ln *k* parameter represents the starting conditions of shoot enlargement (length of the first
219 internode), *a* reflects the rate of shoot elongation, and *b* represents the inhibition process
220 of shoot growth.

221 To test for differences in FA and TA among sites we performed a nested
222 ANOVA using the GLM routine in the statistical program SAS (SAS Institute Inc.,
223 Cary, NC) with the probability of statistical significance set to 0.05 (model III). Site was

224 a fixed factor and the measurement error was removed from the analysis by including
225 the repeated measure as a random factor in the model (Alados and El Aich, 2008).
226
227 2.3.3. Fractal complexity (FC)
228 Fractal complexity was assessed based on branch fractal dimension, which is a measure
229 of plant's efficiency in occupying the space. The higher the IFD, the more efficient the use of
230 space. The photographs of 5–year old branches used to calculate of fractal complexity were
231 digitized using the software Adobe Photoshop version 8.0.1 (Adobe Systems Incorporated).
232 Photoshop 8.0.1 was used to transform the images into raw data, through a process including
233 transform to grayscale, to flatten, to fit threshold, clean others elements different of target plant
234 with eraser and to transform to uniform dimensions (1024 x 1024 pixels). Using the software
235 DRASME 2009, created by J. Escós and C.L. Alados, we calculated the *Information Fractal*
236 *Dimension* (IFD) of each branch (following Alados et al., 1999):

237 $$D_I = \lim_{\varepsilon \to 0} (I_{(\varepsilon)} / \ln(1/\varepsilon)) \quad (4)$$

238 where $I_{(\varepsilon)} = -\sum_{i=1}^{N(\varepsilon)} p_i \times \ln p_i$, and $p_i = \frac{x_i}{\sum x}$, x_i is the number of occupied pixels in

239 each box of size ε. The process was repeated several times using progressively finer
240 grid sizes. $I_{(\varepsilon)}$ was plotted against the log of box size and IFD was defined as the slope
241 of the line (Alados and El Aich, 2008).
242 To tests for differences in branch IFD and biomass among sites, we performed a
243 nested ANOVA. Site was a fixed factor and the other nesting levels were included as
244 random factors. To evaluate the differences in translational asymmetry between the two
245 years we used a repeated measures test because the samples were not independent. TA
246 and shoot length were included in this analysis. We also tested the statistical
247 significance of the interaction term site x year. To assess the statistical significance of

248 the differences among sites in mean values of each variable (FA, TA, *a*, *b*, *k*, IFD and
249 branch biomass) we used a Tukey test.
250
251 3. Results
252 *3.1. Fluctuating asymmetry*
253 At the study sites in northeastern Spain, *Q. faginea* leaves did not exhibit
254 directional symmetry, DS, (F=0.04, p=0.838) or antisymmetry, AS, because although
255 the *L–R* distribution was non–normal (K–S test, p=0.004) the distribution was leptokurtic
256 (skewnes (g1)=0.141, t(g1)=1.906; kurtosis (g2)=1.141, t(g2)=8.335; significance
257 threshold at α=0.05 is t=1.96) (Supporting Information, Fig. S2). Thus, the leaf
258 asymmetry was due to true fluctuating asymmetry, FA (F=28.27, p=0.0001).
259 In 2008 leaf FA differed significantly among sites, and the trees at the mesic site
260 (Arguis) had the highest FA (Table 2, Fig. 2). Leaf development was more stable
261 showing the lowest mean values of the index of leaf response to stress (FA) in the xeric
262 site (Alcubierre) than it was at the mesic and cold sites.
263
264 *3.2. Translational asymmetry (TA).*
265 The repeated measures analyses of TA, a index for developmental instability,
266 showed that the interaction "year by location" was significant (F=8.13, p<0.001) (Fig.
267 3). The same analyses for the variable shoot length showed statistical differences
268 between years and also the interaction "site x year" was significant (F=20.61 and
269 F=20.72 respectively, p<0.001) (Fig. 3). TA values significantly differed among the
270 three sites in both years of the study, but differences were pronounced in 2008 than they
271 were in 2009 (Table 2). Differences in the climatic conditions across the gradient at the
272 three sites influenced DI in *Q. faginea*. In 2008 the curve–fitting was best at the xeric

site (Alcubierre), and worst at the mesic site (Arguis) (Fig. 3). In 2009, however, the best curve fitting was at the cold site (Pico del Águila) and the worst at the xeric site (Fig. 3). In 2009, the Tukey test did not reveal statistically significant differences in TA values among sites.

Shoot length and TA were significant negatively correlated (Kendall correlation (τ) in 2008 (τ=–0.22, p=0.0001), but not in 2009 (τ=–0.06, p=0.112). That is, growth and DI was negative correlated in 2008 but not in 2009. In the models of shoot growth the xeric site (Alcubierre) exhibited the highest *a*, *b*, and ln *k* for in the two years (Fig. 4, Table 3), which indicates that *Q. faginea* at the xeric site had the highest internode elongation rate and the fastest decline at the shoot top. In 2008 all of the fitted parameters except ln *k* differed significantly between the three sites; again, at Alcubierre, the values differed significantly from the values at the other two sites (Tables 2 and 3). In 2009, *b* was the only the parameter that differed significantly among sites (Tables 2 and 3). Thus, shoot growth was more similar among sites in 2009 than in 2008 (Table 2). Evidently, climatic stressors such as drought can influence the rate of shoot growth in *Q. faginea*.

3.3 Fractal complexity (FC).

IFD and branch biomass differed significantly among sites (Table 2); the highest values occurred at the mesic site (Arguis) and the lowest in the xeric site (Alcubierre) for both variables (Table 4). The branch IFD and biomass were strongly correlated and the correlation in the log–transformed data was linear, i.e. data fit a power law function (Fig. 5).

4. Discussion

298 Fluctuating asymmetry and translational asymmetry indices revealed that *Q.*
299 *faginea* trees from the xeric site (Alcubierre) were developmentally more stable after a
300 dry period than were the trees in the other two populations that occurred in more humid
301 and cold areas, which suggest an adaptive response to drought by *Q. faginea*. The shrub
302 *Anthyllis cytisoides* exhibited a similar response (Alados *et al*., 2001). At the semi-arid
303 drought-prone site (Alcubierre), *Q. faginea* trees might have greater resistance to
304 drought stress after the dry period in 2008 than did the individuals in the mesic (Arguis)
305 and cold (Pico del Águila) sites, which are accustomed to having more water available.
306 The individuals at the xeric site exhibited the most rapid internode elongation,
307 probably because of a rapid growth during the short growing season in early spring
308 (Montserrat-Martí et al. 2009). In contrast, the trees at the more mesic sites where more
309 water is available can develop their shoots over a longer period than can those from
310 xeric sites (Alla et al. 2011). Other studies also observed differences in growth rates
311 along aridity gradients (Matesanz et al., 2009; Schilchting, 1986). As expected, after a
312 wet year the shoots of trees at the xeric site behave similarly to those at the sites that
313 had more humid climates resulting in longer internodes and shoots than following a dry
314 periods.
315 The correlation between translational asymmetry and shoot length in *Q. faginea*
316 suggests that there is a trade-off between biomass production (shoot length and branch
317 biomass) and developmental stability when water is scarce. After the dry year in 2008
318 shoot length and TA were negatively correlated; that is, the shorter the shoots, the lower
319 the developmental instability; however, the correlation was not significant after the
320 humid year. Thus, when precipitation is scarce a trade-off between tree growth and
321 maintenance of homeostasis can occur.

After a drought, individuals that were most accustomed to dry conditions shortened their growing period, produced shorter shoots and produced less branch biomass and had lower IFD than did the individuals that were not used to severe and frequent water shortages. That integrative response keeps growth rates relatively low so that developmental stability and homeostasis are maintained. Trees at the mesic sites produced longer shoots, bigger branches and had higher IFD than did the trees at the xeric site, at the expense of higher developmental instability and a change in the allometric relationship between branch biomass and its fractal dimension. After a humid year trees from the xeric site invested their resources in production rather than into maintaining of homeostasis.

Branch fractal dimension is a measure of plant's efficiency in occupying the space, which might reflect how plants are in contact with the environment, as the efficiency in the capture of light and, plausibly, in the diffusion of CO_2 to the atmosphere at the expense of a higher transpiration rate (Foroutan–pour et al., 1999, Foroutan–pour et al., 2001). Conversely a low IFD might reflect a low transpiration rate and a reduced water loss (Alados et al., 2008) at the expense of a reduction in efficient light interception (Horn, 1971). In addition, water stress reduced lateral bud bursting in *Q. faginea*, which affect crown development (Alla et al., 2011; Sanz–Pérez and Castro–Díez, 2010). Apical buds may be favored in order to increase water uptake because they maximize the difference in water potential between the crown and the roots (Lortie and Aarssen, 1997). Thus, trees at the mesic sites, but not those at xeric sites, can maintain a high IFD.

Water availability and temperature during bud organogenesis influence primary growth dynamics of Mediterranean *Quercus* species (Alla et al. 2012). The complex responses in primary growth to climate are the basis for its plasticity and the capacity of

347 Mediterranean oaks to produce more than one growth unit within a single growth period
348 and to produce viable buds of different ages (Barthélémy and Caraglio, 2007). Rainfall
349 can have an immediate effect on shoot elongation depending on whether it occurs
350 during bud organogenesis or shoot elongation (Chaubert–Pereira et al., 2009). The
351 climatic conditions that affect bud formation influence shoot asymmetry, but annual
352 shoot length can be influenced also by the climate of bud development (Chaubert–
353 Pereira et al., 2009). In *Q. faginea* bud enlargement occurs in August–September of the
354 year before shoot elongation (Alla et al., 2012) and typically bud bursting occurs from
355 March to April (Montserrat–Marti et al., 2009). In humid locations like Arguis,
356 however, it can occur slightly later (Sanz–Pérez, 2010), which may be why in Arguis
357 shoots were longer in 2008 than in 2009. In Arguis bud enlargement period in 2009 was
358 drier and colder than the average, which probably shortened the spring growing season
359 when shoots elongate (see appendix for climate data). Furthermore, Alla et al. (2011)
360 reported similar shoot lengths in the same years at the same study sites.
361 Water uptake is critical for primary growth in drought–prone areas. Turgor
362 pressure limits cell enlargement and consequently cell division (Hsiao et al., 1976). In
363 addition, drought limits photosynthesis and carbon uptake through stomatal closure and
364 a reduction in ribulose biphosphate carboxylase/oxidase activity (Flexas and Medrano,
365 2002), which might be why the shortest shoots found in the *Q. faginea* trees from the
366 xeric Alcubierre site. Furthermore, stressed plants tend to show decreased growth
367 because it reduces the demand for water and nutrients (Grime, 1977). Changes in plant
368 size involve shifting priorities among growth types (e.g., shoot elongation *vs.* shoot
369 thickening) and changing the allocation priority of resources within the plant (water,
370 nutrients, carbohydrates) (Tilman, 1988). A reduction in growth might drive more
371 resources into assimilating organs (leaves) and fewer into supporting tissues (wood)

372 which increases the likelihood of survival in harsh xeric environments (Chapin, 1991).
373 In addition, the phenotypic expression of traits that are functionally important to the
374 organism, such as the allometric relationships between organs or leaf symmetry,
375 influence plant fitness (Alados et al., 2001).

376 The behavior of *Q. faginea* at the xeric site in northeastern Spain is consistent
377 with Levitt (1972) concept of "*resistance adaptation*" as an explanation for how plants
378 adapt to a high intensity stress event after having been subjected to the same stress,
379 previously, but a lower intensity ("***capacity adaptation***"). There is a climatic threshold
380 at which "*resistance adaptation*" is triggered in those individuals best adapted to
381 changes in climatic conditions. Several studies have demonstrated that responses
382 thresholds to environmental changes exist in plants (Bielorai, 1973; Razzahi, 2011), and
383 that a minimum threshold of a climatic factor is needed to trigger a growth response in
384 trees (Deslauriers et al., 2008; Levitt, 1972). Even gradual changes in environmental
385 conditions can induce sharp responses in trees; e.g., the way they use water in semi-arid
386 ecosystems (Williams and Ehleringer, 2000). The drought in Alcubierre triggered a
387 conservative strategy in *Q. faginea*, there was a point within the continuous variation in
388 environmental conditions through the time when *Q. faginea* developed an adaptive
389 response.

390 In Alcubierre, how did *Q. faginea* adapt to the variability and uncertainty in
391 water availability? Morphological and physiological tradeoffs prevent plants from being
392 optimally adapted to both dry and wet conditions (Schwinning and Ehleringer 2001).
393 Adaptive response thresholds might be common in species that depend on fluctuating
394 resource supply, as soil water in semi-arid areas, because a threshold response involves
395 adaptations to minimize the cost-to-benefit ratio of resource use (Schiwinning and Sala,

2004). To our knowledge our study is one of the few that have demonstrated an adaptive response threshold in trees.

In our study, the interaction between 'year' and 'location' on TA and shoot length demonstrate that trees from the xeric site are able to tolerate the environmental stress imposed by a severe water shortage. The flexibility of that trait in response to the environmental change reflects the adaptive phenotypic plasticity of *Q. faginea*. Other studies have shown that phenotypic plasticity in woody plants such as shrubs can be an adaptive response to a local climatic constraint (Bedetti et al, 2011).

An understanding of the adaptive phenotypic plastic responses to changes in environmental conditions is important because inter–annual variability in weather is expected to increase as a result of climate change which means that severe droughts might become more frequent in the Mediterranean Basin (Giorgi and Lionello 2008). Long–lived sessile organisms such as trees might experience rapid climate change along one or two generations and may do not have enough time to evolve responses to rapidly changing conditions (Fallour–Rubio et al., 2009; Lindner et al.2010).

We conclude that *Quercus faginea* can generate an adaptive response to drought in xeric environments. If the pace of climate change is faster than the individuals' ability to adapt, trees will not be able to produce a plastic response and will exhibit developmental errors in the shape of their crown. Developmental instability can be used as an indicator of stress, and it can indicate a degree of adaptiveness of the species to specific environmental conditions. Fluctuating asymmetry reflects the degree of adaptation of a population to site conditions (Graham et al., 2010). As an index of adaptation developmental instability can be used to estimate the viability of a tree population, to detect adaptive changes or shifts in organisms, and to identify the environmental conditions that lead to adaptive responses. Developmental instability can

421 be used as an indicator of the adaptive ability of a tree species to specific environmental
422 conditions and as an estimator of threshold adaptive responses by measuring several
423 growth characters during years that have contrasting climatic conditions.
424
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641 Table captions

642

643 Table 1. Characteristics of the studied sites, years and *Q. faginea* trees in northeastern
644 Spain. Climate data were obtained from nearby meteorological stations collected in the
645 period 1960–2006 (see Alla et al. 2011 and Alla and Camarero 2012).

646

647 Table 2. Statistical parameters derived from nested ANOVAs of stress indicators (FA,
648 R^2, *a*, *b*, ln *k*, IFD, branch biomass) in *Q. faginea* among study sites and years (2008,
649 2009) (*F* values and, in brackets, the degrees of freedom). Significance levels:
650 $^{**}p<0.0001$, $^{*}p<0.05$.

651

652 Table 3. Values (mean ± SE) of the growth parameters (*a*, *b*, ln *k*) in *Q. faginea* for the
653 three study sites and the two study years (2008, 2009). Different letters show significant
654 ($P<0.05$) differences among sites in each year of study based on Tukey tests.

655

656 Table 4. Values (mean ± SE) of *Information fractal dimension* (IFD) and branch
657 biomass of 5–year old branches of *Q. faginea* in 2008 at the three study sites, Pico del –
658 Águila (cool site), Arguis (mesic site) and Alcubierre (xeric site).

659

660 Figure captions

661

662 Figure 1. Leaf and a current–year shoot of *Q. faginea*. In the leaf, A–B represents the
663 central axis, C–G, E–I and D–K and C–F, E–H and D–J represent right and left measures,
664 respectively, used to calculate fluctuating asymmetry. In the shoot S1, S2, etc. represent
665 the lengths of each internode (distance between successive non–apical or lateral buds)
666 used to calculate translational asymmetry.

667

668 Figure 2. Values of the index of stress (*FA*) for each study site. Different letters show
669 significant (P<0.05) differences between sites (Tukey test). Means ± SE (n=1080).

670

671 Figure 3. Mean R^2 (a) and mean shoot length (b) of *Q. faginea* in two years at three
672 sites in northeastern Spain. Different letters show significant (P<0.05) differences
673 between sites based on Tukey tests.

674

675 Figure 4. Estimated internode lengths of *Q. faginea* shoots in two years at three sites in
676 northeastern Spain that differed in climate as a function of node order derived from the
677 equation, $L(N) = kN^a e^{-bN}$.

678

679 Figure 5. *Information Fractal Dimension* (IFD) and branch biomass of *Q. faginea*
680 branches at the three study sites. The fitted regression to log–transformed values of both
681 variables was highly significant ($R^2 = 0.92$, p<0.001).

682

683

684

685 Supporting Information

686

687 Figure S1. Relevant concepts in the study and in Developmental Instability theory, their
688 abbreviation, definition, goal and references.

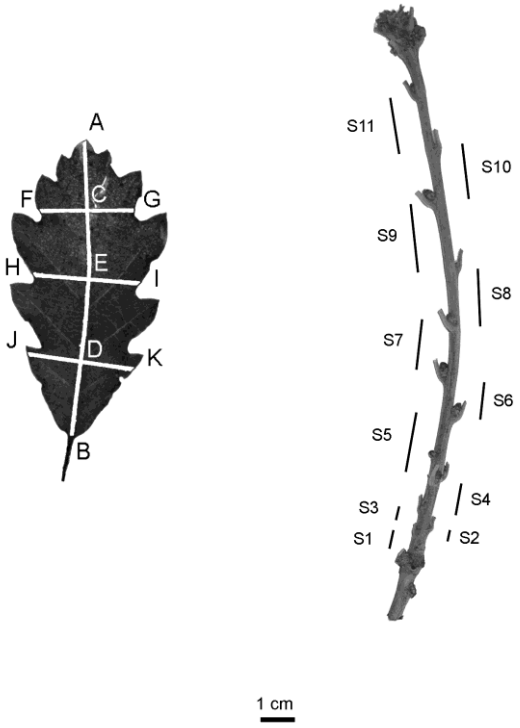
689

690 Figure S2. Histogram of the distribution of left minus right (*L–R*) sides of *Q. faginea*
691 leaves.

692

693 Figure S3. Ombrothermic diagram of temperature (ºC) and precipitation (mm) in
694 Alcubierre and Argis in the years that influenced the shoots development, 2007, 2008
695 and 2009, and the reference period.

696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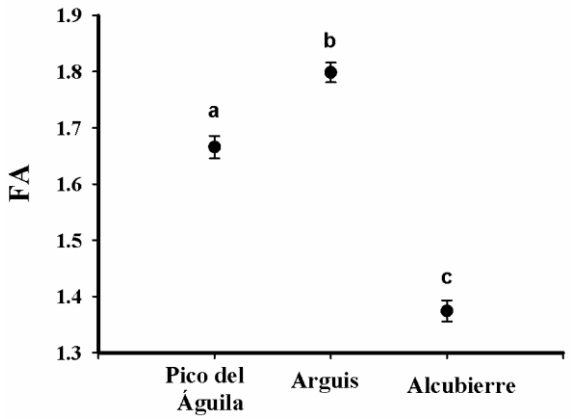


Figure 2

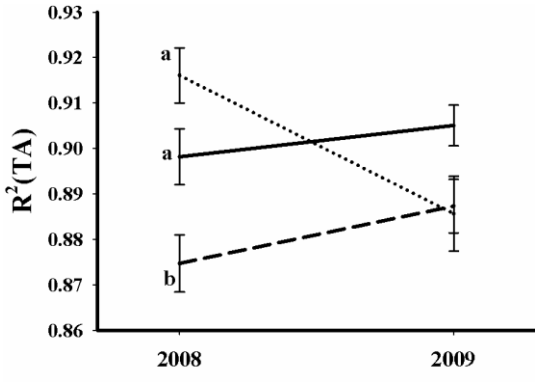


Figure 3a.

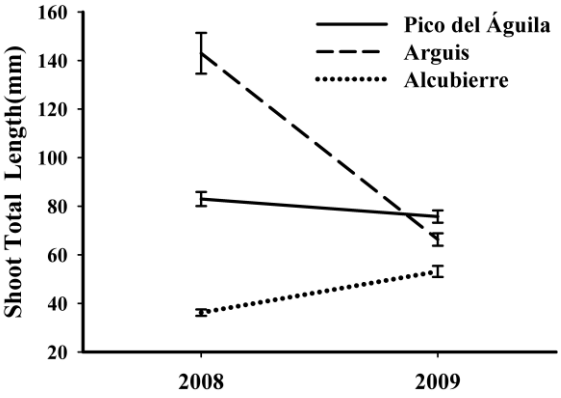
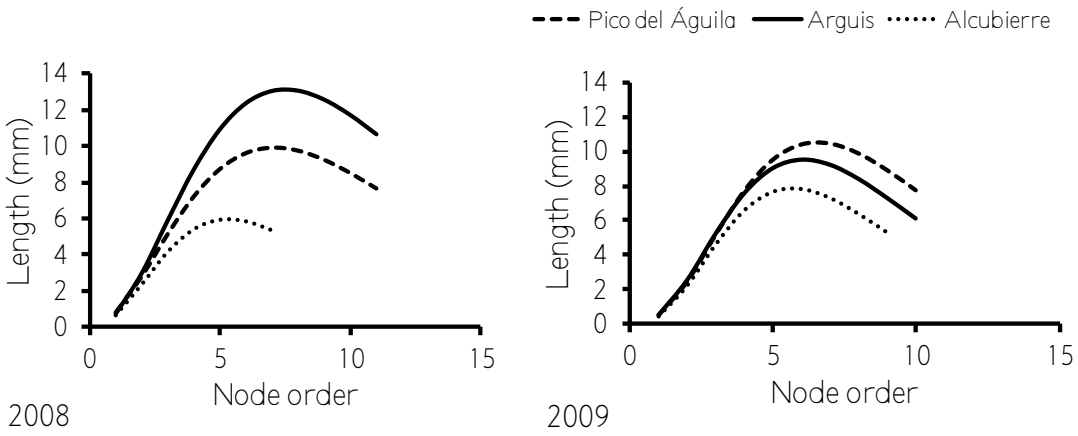
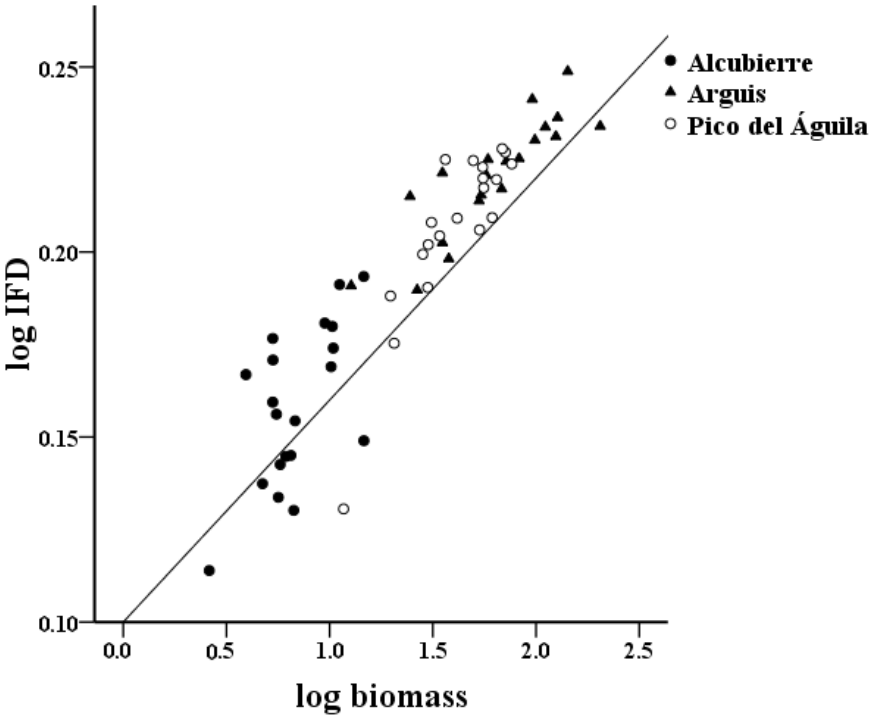


Figure 3b.


Pico del Águila
Arguis
Alcubierre
Length (mm)
Node order
2008
2009


log IFD
0.25
0.20
0.15
0.10
0.0
0.5
1.0
1.5
2.0
2.5
log biomass
Alcubierre
Arguis
Pico del Águila

Site (type)	Altitude (m)	Diameter at 1.3 m (cm)	Mean temperature (°C)			Total precipitation (mm)		
			2007/ August–July **	2008/ August–July **	Reference period [1960–2006]	2007/ August–July**	2008/ August–July **	Reference period [1960–2006]
Pico del Águila (cold site)	1490	9.2 ± 0.8	7.03/ 7.40	7.02/ 6.95	6.1	933/1271	1464/1183	1215
Arguis (mesic site)	1140	15.9 ± 1.0	8.83/ 9.30	8.82/ 8.73	7.2	646/880	1010/816	849
Alcubierre (xeric site)	650	12.0 ± 1.5	11.43/ 12.04	11.42/ 11.30	10.8	350/477	564/456	540

**Period including part of bud preformation and primary growth from August 2007 up to July 2008, and from August 2008 up to July 2009, respectively. Note that the reference period was calculated considering Julian years.

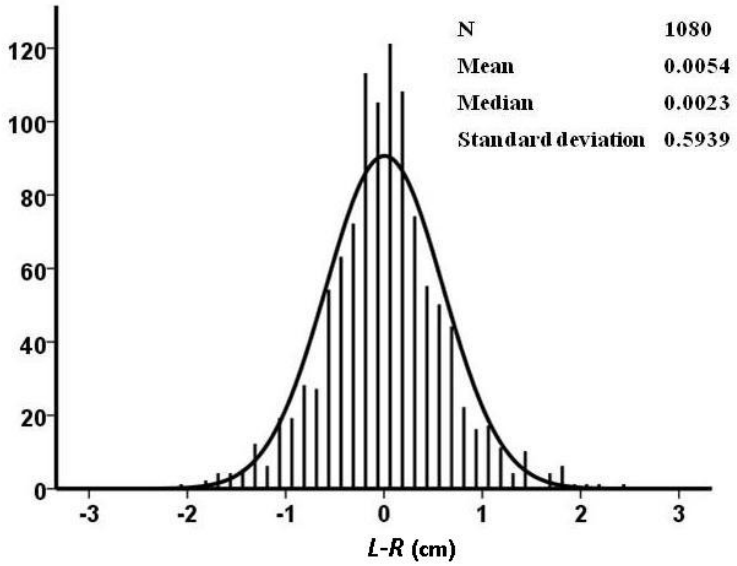
	Site	Transect (Site)	Individual (Transect)	Shoot (Individual)	Leaf (Shoot)	Repet (Leaf/Shoot)
Fluctuating asymmetry						
FA	56.57** (1023,2)	8.29* (1023,2)	1.18 (1023,9)	1.37 (1023,18)	1.29 (1023,4)	1.17 (1023,3)
Translational asymmetry						
2008						
TA	12.29**(313,2)	0.24 (313,2)	2.87*(313,9)	1.55 (313,18)	---	1.12 (313,3)
a	7.29*(313,2)	0.58 (313,2)	1.24 (313,9)	1.00 (313,18)	---	1.00 (313,3)
b	25.76** (313,2)	0.17 (313,2)	1.45 (313,9)	1.66 (313,18)	---	0.07 (313,3)
ln *k*	0.46 (313,2)	3.94* (313,2)	0.76 (313,9)	2.11* (313,18)	---	5.79**(313,3)
2009						
TA	3.14*(311,2)	3.01 (311,2)	2.50* (311,9)	1.64* (311,18)	---	0.20 (311,3)
a	2.33 (311,2)	0.22 (311,2)	2.51* (311,9)	1.00 (311,18)	---	0.12 (311,3)
b	5.24* (311,2)	0.39 (311,2)	2.23* (311,9)	1.11 (311,18)	---	0.09 (311,3)
ln *k*	0.21 (311,2)	1.57 (311,2)	2.03* (311,9)	1.29 (311,18)	---	0.42 (311,3)
Fractal complexity						
IFD	49.41** (36,2)	0.91 (36,2)	0.88(36,18)	---	---	---
Biomass	29.91** (36,2)	3.93* (36,2)	1.04(36,18)	---	---	---

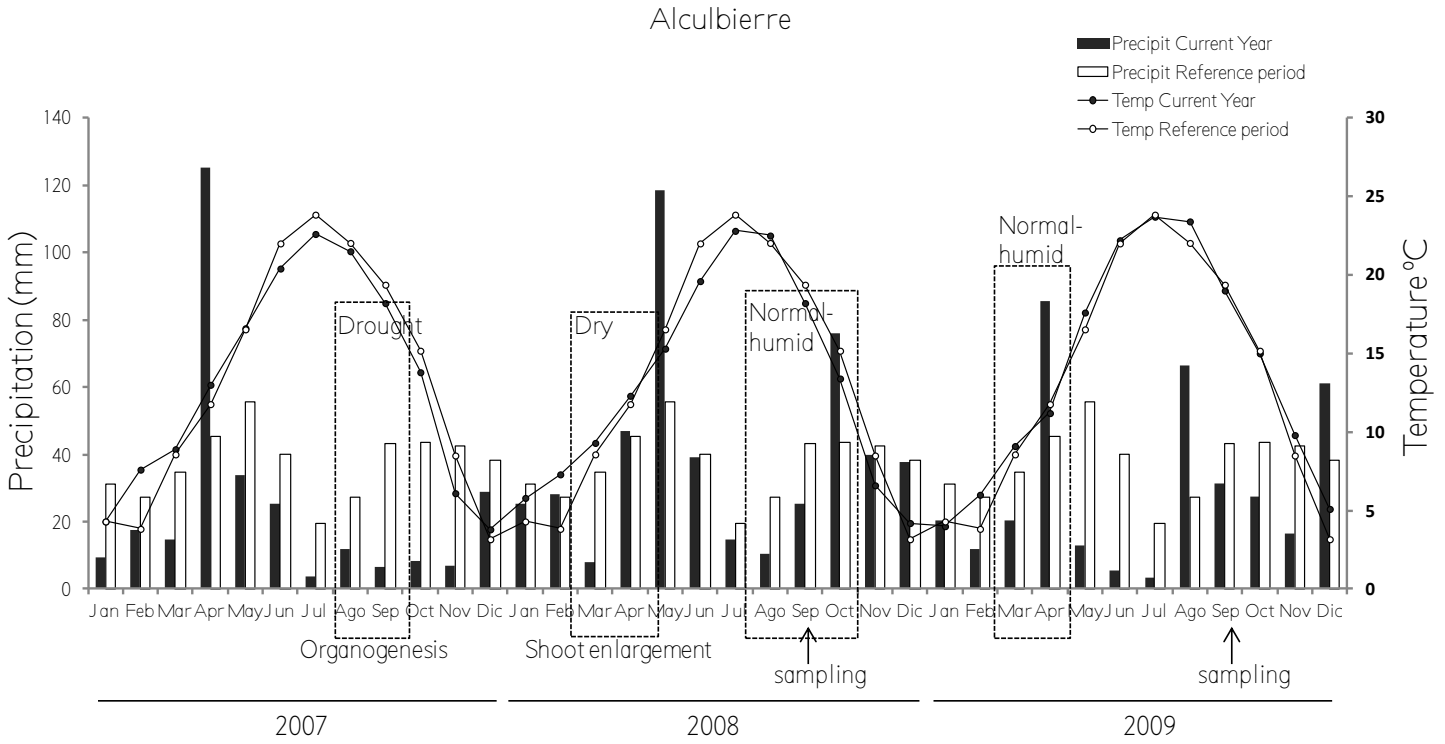
Growth parameters	Pico del Águila (cold site)	Arguis (mesic site)	Alcubierre (xeric site)
2008			
a	2.324 ± 0.594^{b}	2.517 ± 0.677^{ab}	2.646 ± 0.747^{a}
b	0.327 ± 0.147^{b}	0.335 ± 0.170^{b}	0.498 ± 0.295^{a}
ln *k*	0.065 ± 0.378	0.012 ± 0.584	0.006 ± 0.614
2009			
a	2.981 ± 0.644	2.994 ± 0.961	3.210 ± 1.163
b	0.455 ± 0.160^{b}	0.492 ± 0.264^{ab}	0.565 ± 0.407^{a}
ln *k*	0.271 ± 0.436	0.163 ± 0.472	0.309 ± 0.662

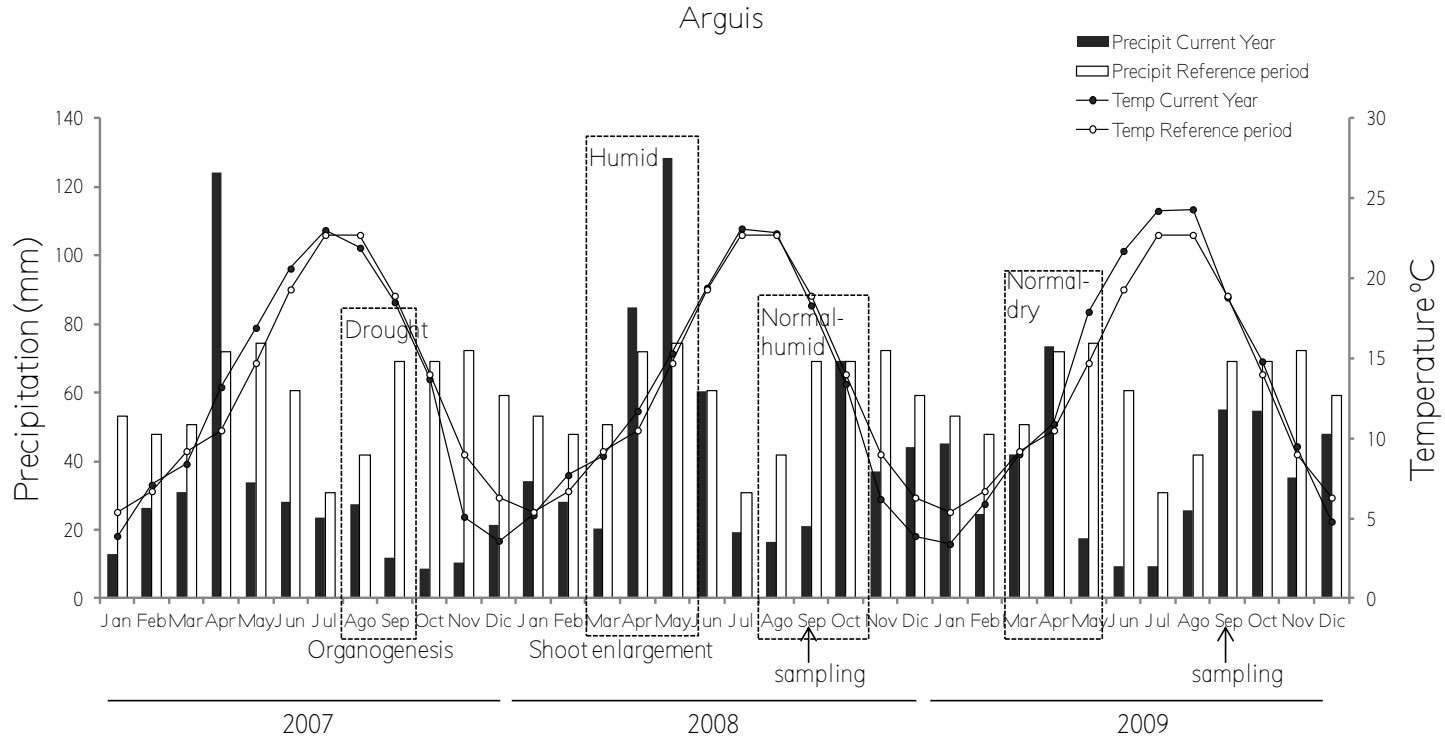
	Pico del Águila	Arguis	Alcubierre
IFD	1.611 ± 0.081	1.664 ± 0.061	1.44 ± 0.071
Biomass (g)	44.68 ± 18.83	76.09 ± 47.96	7.55 ± 3.37

Concept	Abbr.	Definition	Goal	References
Developmental instability	DI	The error in the development of organism due to genetic or environmental stress.	Traditionally used as index of stress	• Møller & Swaddle. 1997. Asymmetry, Developmental Stability and Evolution. OUP. • Polak. 2003. Developmental instability. OUP.
Developmental stability	DS	The capacity of organisms to maintain their development.	DI theory	• Moller & Shykoff. 1999. Int. J. Plant Sci. 160, 135–146. • Polak. 2003. Developmental instability. OUP.
Developmental noise	DN	The error in the development due to the non-linear nature of cellular processes.	DI theory	• Lens et al. 2002. Biol. Rev. 77, 27–38.
Translational asymmetry	TA	The error in the allometric relationship between internode length and node order. Is the asymmetry of shoots.	DI measure	• Alados, Navarro, Cabezudo, Emlen & Freeman. 1998. Evol. Ecol. 12, 21–34. • Freeman, Brown, Duda, Graham, Emlen, Krzysik, Balbach, Kovacic & Zak. 2004. Int. J. Plant. Sci. 165, 53–63.
Fluctuating asymmetry	FA	The bilateral asymmetry. The distribution of the L–R is normal with mean 0.	DI measure	• Palmer & Strobeck. 1986. Annu. Rev. Ecol. Syst. 17, 391–421. • Swaddle, Witter &Cuthell. 1994. Anim. Behav. 48, 986–989. • Graham, Emlen, Freeman, Leamy & Kieser. 1998. Biol. J Linn. Soc. 64, 1–16.

Directional symmetry	DS	Presented in organisms that are genetically determined to be asymmetric. All the individuals have one side greater than the other. The distribution of L–R is normal but the mean is not 0.	DI methodology	• Palmer & Strobeck. 1992. Acta Zool. Fennica. 191, 57–72.
Antisymmetry	AS	Presented in organisms that are genetically determined to be asymmetric. Some organisms have one side greater and others organisms the other. The L–R distribution is platikurtic o bimodal.	DI methodology	


N 1080
Mean 0.0054
Median 0.0023
Standard deviation 0.5939
120
100
80
60
40
20
0
-3
-2
-1
0
1
2
3
L-R (cm)


Alculbierre
Precipit Current Year
Precipit Reference period
Temp Current Year
Temp Reference period
Precipitation (mm)
Temperature °C
Drought
Dry
Normal-humid
Normal-humid
Organogenesis
Shoot enlargement
sampling
sampling
2007
2008
2009


Arguis
Precipit Current Year
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Temperature °C
Drought
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